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TECHNICAL MANUSCRIPT 106

EVOLUTION OF ETHYLENE  
FROM EXCISED ABSCISSION ZONES

DECEMBER 1963

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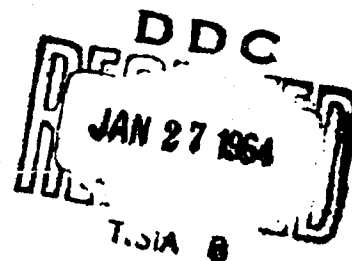
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TECHNICAL MANUSCRIPT 106

EVOLUTION OF ETHYLENE FROM EXCISED ABSCISSION ZONES

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Project 1A013001A039

December 1963

This material was originally submitted as Manuscript 5239.

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# ABSTRACT

Ethylene evolution from abscission zone explants has been measured with a gas chromatograph. Naphthaleneacetic (NAA) causes marked increases in the amount of ethylene produced regardless of the abscission stage to which it is applied. Additions of ethylene to explants show that the gas is active only during the second abscission stage. Ethylene present at this stage may account for the auxin stimulation of abscission. Ethylene produced by immediate auxin applications is unable to stimulate abscission because auxin simultaneously retains the explants in the first stage of abscission.

## I. INTRODUCTION

Ethylene additions have long been known to markedly stimulate leaf abscission.<sup>1</sup> Evolution of ethylene has been detected from leaves,<sup>2,3</sup> but little has been done to correlate the presence of this gas with the onset of natural leaf abscission. The results presented here will trace the evolution of ethylene from abscission zone explants and compare the presence of physiologically active amounts of the gas with time of abscission.

## II. MATERIALS AND METHODS

The experimental materials consisted of one-centimeter excised abscission zones cut from primary leaf petioles of ten-day-old bean plants (*Phaseolus vulgaris* L. var. Red Kidney) grown at  $26^{\circ} \pm 2^{\circ}\text{C}$  and 1200 foot-candles with a 12-hour photoperiod. Each explant was composed of the abscission zone with five millimeters of tissue at the distal side and five millimeters of petiole at the proximal side. Six milliliters of agar were poured into 42-ml gas collection bottles that were fitted with gas-tight vaccine caps. Ten explants were then inserted into the agar so that three millimeters of the proximal ends were submerged. When desired, auxin was applied proximally by incorporation into the agar. The bottles were subsequently incubated at  $16^{\circ}\text{C}$  and 400 foot-candles of continuous light.

Gas samples were removed with a syringe and analyzed for ethylene by an F & M Model 720 gas chromatograph equipped with a flame ionization detector. A two-foot activated alumina column was used at  $45^{\circ}\text{C}$ ; helium flow rate was 110 milliliters per minute. Data are expressed as millimicroliters (m $\mu$ l) of ethylene evolved per explant but can be converted roughly to parts per million by dividing by 3.4 or expressed as m $\mu$ l per milligram dry weight by dividing by 2.71. The sensitivity of the method permitted accurate measurements of ethylene as low as 0.15 m $\mu$ l per explant per 12 hours using a two-milliliter sample.

Authenticity of the ethylene peak was determined by co-chromatography with an ethylene standard. Additional confirmation was obtained by using mercuric perchlorate and sodium chloride as specific reagents for the absorption and release of ethylene.<sup>4</sup>

### III. RESULTS

To determine if detectable quantities of ethylene were evolved from naturally abscising explants, two-milliliter gas samples were withdrawn from vented and unvented gas collection bottles. Bottles were vented every 12 hours to remove any accumulated ethylene. Different sets of gas collection bottles were used for the unvented treatments so that the internal pressures would remain equal during the course of the experiments. Figure 1 shows that ethylene was evolved from both vented and unvented bean explants. The gas was produced rapidly for 24 hours and then continued linearly until the end of the experiments. Ethylene production by vented explants (Figure 1) shows clearly an initial ethylene burst, but after 24 hours less than 0.15 millimicroliter of the gas per explant could be detected. The initial evolution of ethylene may be similar to the wounding response described by Williamson<sup>5</sup> and Burg.<sup>6</sup> The unvented treatments, which ultimately contained larger amounts of ethylene, reached 50 per cent abscission at 50 hours and 100 per cent abscission at 72 hours; 50 per cent of the vented explants abscised at 80 hours.

Rubinstein and Leopold<sup>7</sup> have shown that bean leaf abscission could be separated into two stages — an initial stage when abscission was inhibited by  $\alpha$ -naphthaleneacetic acid (NAA) and a later stage when NAA stimulated abscission. The effect of NAA on ethylene production during these two stages was investigated, using gas collection bottles that were vented every 12 hours (Figure 2). If explants were placed in NAA at either 0 or 24 hours after cutting, abscission was inhibited and large amounts of ethylene were evolved. Insertion of explants into NAA 48 hours after cutting stimulated abscission, but slightly less ethylene was detected as compared with those placed in NAA immediately. Similar results were obtained with indoleacetic acid. One must conclude from these data that if ethylene is directly involved in accelerating explant abscission, its stimulatory effect is counteracted by the presence of auxin, which retains the explants in the first abscission stage.<sup>7</sup>

Attempts were then made to determine the effect of ethylene on the two stages. This was done by injecting known quantities of the gas into gas collection bottles either immediately after placement of the explants or at 48 hours after placement; all treatments were vented at 12-hour intervals. The results in Table I indicate that a 12-hour exposure of 0.34 to 3.40  $\mu$ l of ethylene per explant was ineffective in stimulating abscission if applied immediately. When, however, the explants were exposed to the same concentrations of the gas at 48 to 60 hours after cutting, 50 per cent abscission occurred 20 hours sooner than in the controls. These data suggest that ethylene, like amino acids,<sup>7</sup> stimulates abscission only during the second stage.

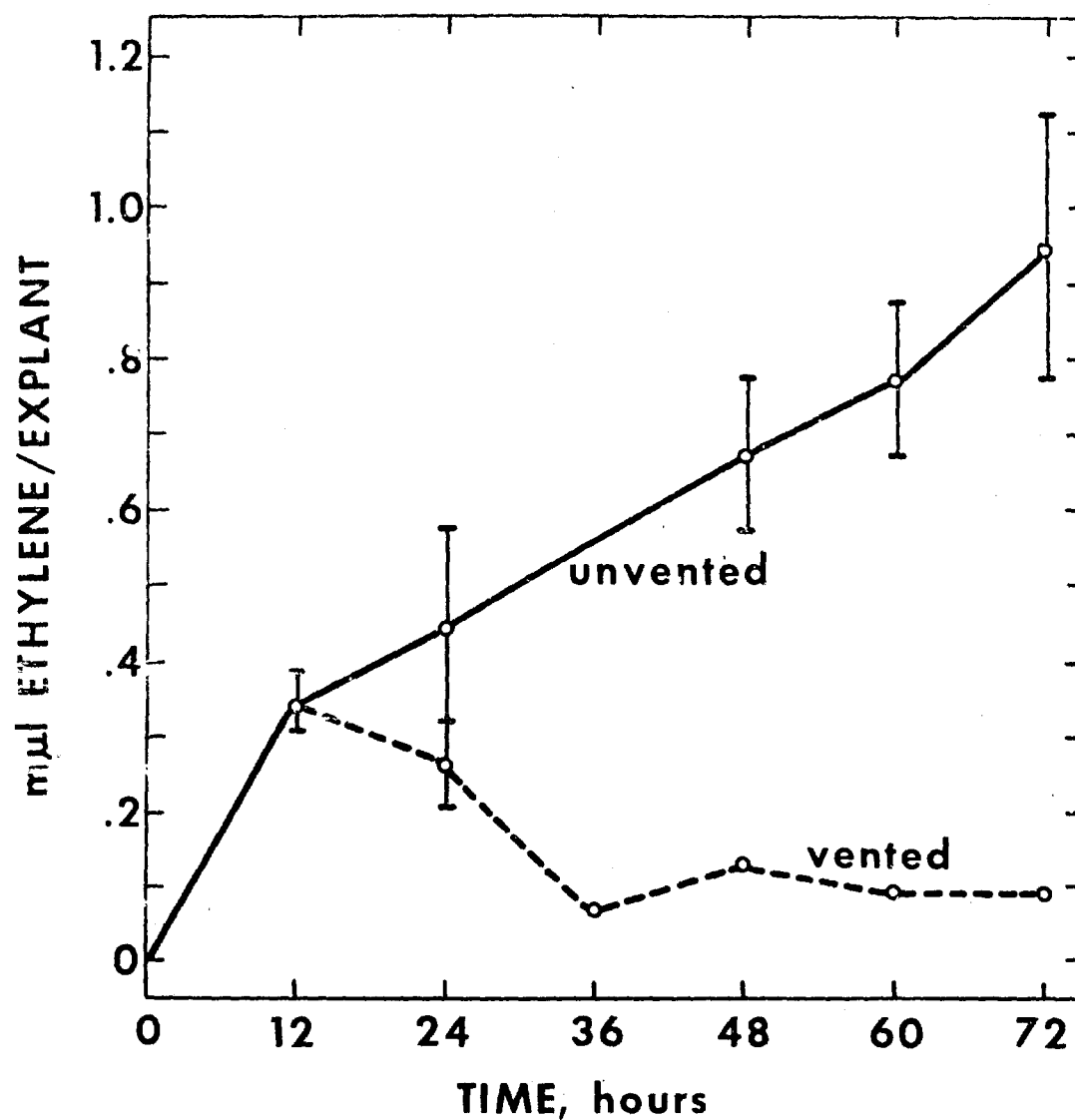


Figure 1. Production of Ethylene by Unvented and Vented Bean Explants. Unvented explants reached 50 per cent abscission in 50 hours; unvented explants abscised at 80 hours. The line through each point represents standard error of the mean.

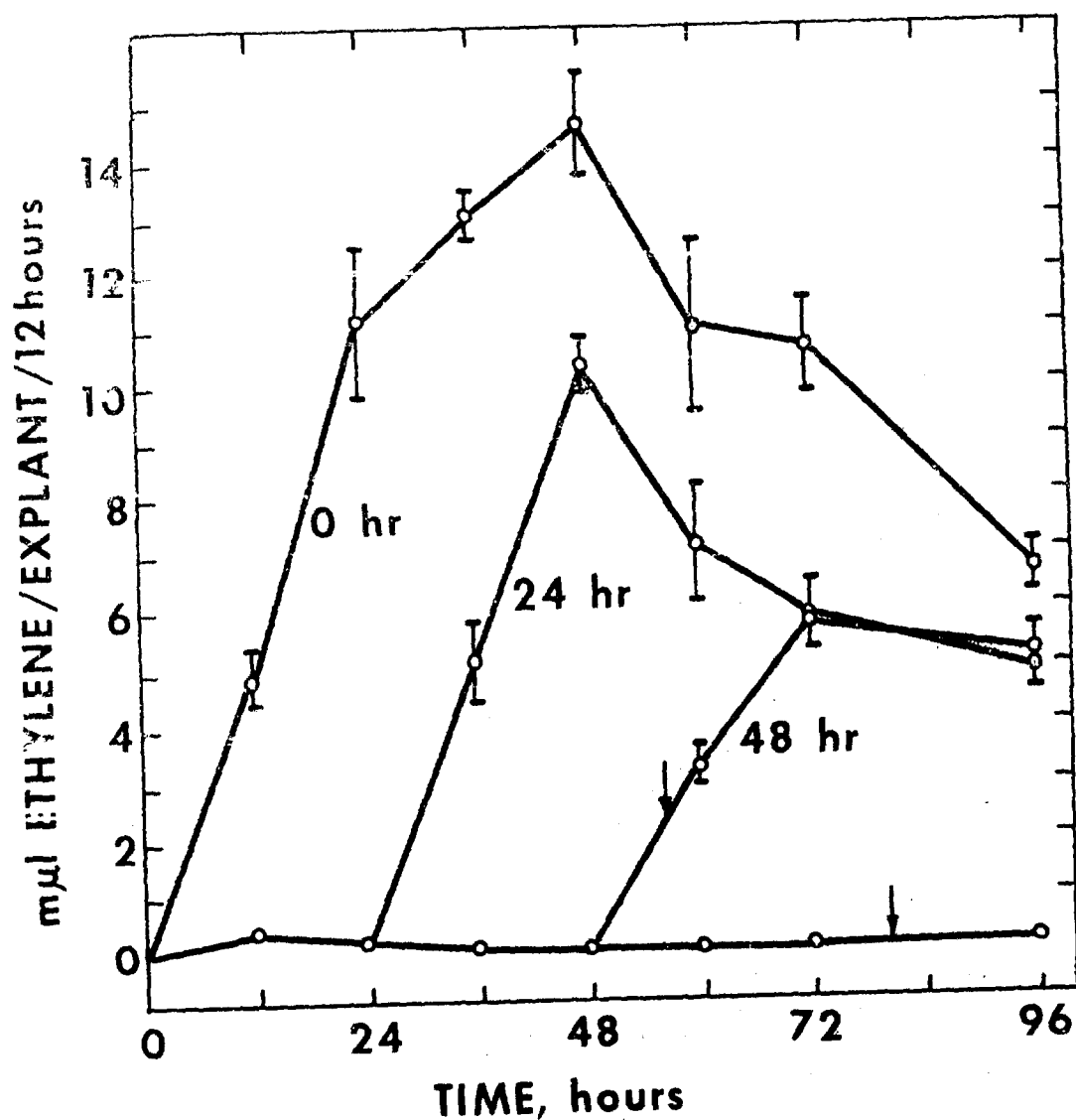


Figure 2. Effect of  $\alpha$ -Naphthaleneacetic Acid (NAA) Applications at Various Intervals After Cutting on the Production of Ethylene by Vented Bean Explants. Vertical arrows indicate hours to 50 per cent abscission. No abscission was observed for explants placed in NAA immediately or after 24 hours. The line through each point represents standard error of the mean.

TABLE I. HOURS TO 50 PER CENT ABSCISSION OF  
BEAN EXPLANTS AFTER ETHYLENE EXPOSURES  
AT 0 OR 48 HOURS

Ethylene Concentration, $\mu$ l	Time of Exposure, hours <sup>a/</sup>	
	0 to 12	48 to 60
0.0	80 $\pm$ 3	
0.34	78 $\pm$ 5	58 $\pm$ 2
1.70	79 $\pm$ 7	56 $\pm$ 2
3.40	78 $\pm$ 3	55 $\pm$ 2

a. Data represent hours to 50 per cent abscission with their standard errors.

In our experiments, the amounts of ethylene present per explant during the second stage were 0.6 to 0.8  $\mu$ l for unvented explants in plain agar (Figure 1) and 3.5  $\mu$ l for explants inserted in NAA after 48 hours (Figure 2). Since these concentrations of ethylene can induce abscission when present during stage two (Table I), we feel that endogenously produced ethylene may be important for induction of abscission in bean explants. The vented explants (Figure 1) contain less than 0.15  $\mu$ l of the gas per explant at 48 hours and abscise later than unvented treatments, suggesting that ethylene levels were suboptimal for a rapid ethylene stimulation of abscission. Before an ethylene stimulation can occur, however, the explants must be in the second stage as measured by the increased rate of abscission upon contact with NAA. Thus, even though immediate applications of NAA stimulate the production of high amounts of ethylene, the gas is unable to promote abscission because the NAA simultaneously retains the explants in the first abscission stage.

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